


**DATA EVALUATION RECORD**  
**ACUTE (SINGLE DOSE) TOXICITY TESTS WITH THE HONEY BEE LARVAE**  
**NON-GUIDELINE**


1. **CHEMICAL:** Ipconazole PC Code No.: 125618
2. **TEST MATERIAL:** Ipconazole (technical material) Purity: Purity: 96.7% w/w  
(as total Ipconazole),  
89.7% w/w (as Ipconazole cc),and  
7.0% w/w (as Ipconazole ct)
3. **CITATION**

Authors:	Patnaude, M. R.
Title:	Ipconazole - Honey Bee ( <i>Apis mellifera</i> ) Larval Toxicity Test, Single Exposure
Study Completion Date:	October 31, 2017
Laboratory:	Smithers Viscient
Sponsor:	Kureha Corporation
Study No.:	11106.610
MRID:	50451001
DP Barcode:	450645
4. **REVIEWED BY:** Holly Dimig, Junior Staff Scientist, CDM/CSS-Dynamac JV  

Signature: 

Date: 05/01/2019

**APPROVED BY:** Adrian Graff, Environmental Scientist, CDM/CSS-Dynamac JV  

Signature: 

Date: 05/13/2019
5. **APPROVED BY:** Holly Rogers, Biologist, EPA/OPP/EFED/ERB5  

Signature:

Date: 05/04/2020

**APPROVED BY:** Hannah Yingling, Biologist, USEPA/EPA/OPP/EFED/ERB5

**Signature:** *Hannah B. Yingling***Date:** 04/28/2020

*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.*

## 6. STUDY PARAMETERS:

**Test Species and strain:** Honey bees (*Apis mellifera*)

**Age of Test Organisms at Test Initiation:** 1<sup>st</sup> instar larvae, ≤24 hours old at grafting

**Exposure Duration:** 72 hours (3 days after application)

## 7. CONCLUSIONS:

Individual synchronized honey bee (*Apis mellifera*) larvae (first instar, ≤24 hours old), were exposed *in vitro* to Ipconazole (technical material) at the nominal diet concentrations and doses reported in the table below. All stock solution, royal jelly diet, and QC samples were analyzed for total Ipconazole concentrations (as the sum of the Ipconazole cc and ct isomers, which were quantified separately). The study author calculated the mean-measured concentrations from analytical verification of the test item in the larval diet. Recoveries were used in tandem with the mass of diet provided to each larva to calculate the measured doses. The measured concentrations and doses are summarized in the table below:

Nominal Concentration (µg ai/g diet)	Mean Measured Concentration (µg ai/g diet)	Nominal Dose (µg ai/larva)	Measured Dose (µg ai/larva)
180	170	6.3	5.7
380	360	13	12
730	730	25	25
1500	1400	50	49
3000	3100	100	100

Larvae used in the study were obtained from David Wood, Forsyth, Georgia. A negative control and a solvent control were utilized and Dimethoate was used as a reference toxicant at 8.8 µg ai/larva. All groups consisted of 3 replicates with 12 larvae/replicate for a total of 36 larvae. Each larva was contained within a single grafting cell that was within a 48-well cell culture plate. On Day 4, the larvae were exposed to treated diet or untreated control diet. On Days 5 and 6, larvae were provided untreated artificial diet. Survival was assessed daily from Day 4 through 7. Uneaten diet and health observations were recorded at test termination, Day 7.

At test termination (Day 7), cumulative larval mortality was 0% in both the negative and solvent controls, respectively, as compared to mortality ranging from 8 to 100% in the treatment groups. On Day 7, 1 larva in the control and 3 larvae in the solvent control were observed to be small in size, as compared to 4, 4, 7, and 4 larvae observed as small in size in the measured 5.7, 12, 25, and 49 µg ai/larva doses, respectively. All other surviving larvae fed treated and control diets were observed to be healthy.

On Day 7, uneaten food was present in 11 replicates in both the negative and solvent controls, as compared to uneaten food present in 13, 12, 12, and 4 replicates in the 5.7, 12, 25, and 49 µg ai/larva doses, respectively.

Based on these results, the NOAEC and 72-hour LC<sub>50</sub> were 170 and 649 µg ai/g diet, respectively, corresponding to a NOAEL and LD<sub>50</sub> of 5.7 and 22 µg ai/larva, respectively.

The study **is scientifically sound** and is classified as **acceptable** for measuring acute (single dose) toxicity to honeybee larvae.

	<b>Mortality</b>
Diet Concentration (µg ai/g diet)	LC <sub>50</sub> : 649 95% CI: 540 to 779 Slope: 3.1 (2.4 to 3.9) NOAEC: 170 LOAEC: 360
Dietary Dose (µg ai/larva)	LD <sub>50</sub> : 22 95% CI: 18 to 27 Slope: 3.1 (2.4 to 3.8) NOAEL: 5.7 LOAEL: 12

## 8. ADEQUACY OF THE STUDY:

**A. Classification:** This study **is scientifically sound** and is classified as **acceptable**.

**B. Rationale:** There were no major deviations or deficiencies in this study and both validity criteria for the OECD guideline used were met in this study:

- 1) Cumulative larval mortality from D4 to D7  $\leq$  15% across control replicates.
- 2) Larval mortality in the reference chemical treatment (Dimethoate) was  $\geq$  50% at D7.

**C. Reparability:** N/A

9. **GUIDELINE DEVIATIONS:** This study was performed following a Smithers Viscient protocol based on the OECD Guideline 237, Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013). The following deviations from OECD Guideline 237 were noted by the reviewer:

- 1) Royal jelly diet preparation ratios of Diets A, B, and C are based on modifications from Schmehl *et al.* (2016). The composition of diets A and B deviated from OECD guidance.
- 2) The shape of the grafted larvae at transfer was not reported. OECD recommends that newly hatched larvae are selected that have not yet formed a “C” shape.
- 3) The temperature was slightly below the suggested range.

Days 1 to 7: 33-34 °C (recommended 34-35 °C)

- 4) Three replicates each containing 12 larvae per treatment level and control group were utilized (following OECD Guidelines). According to U.S. EPA’s “Honeybee Toxicity Testing Frequently Asked Questions – August 16, 2018”, the individual larva is considered the replicate based on each single larva receiving an individual dose and being housed in a separate well.

These deviations **do not** affect the study.

10. **SUBMISSION PURPOSE:** To determine the effects on mortality and sub-lethal effects of Ipconazole on the honey bee (*A. mellifera*) larvae from acute [single dose] exposure following the OECD Test Guideline 237 (2013) for the purpose of chemical re-registration.

11. **MATERIALS AND METHODS:**

**A. Test Material**

Test Material:

Synonym:

IUPAC Name:

Description:

Lot No./Batch No.:

Purity:

Ipconazole (technical material)

Not reported

(1RS,2SR,5RS;1RS,2SR,5SR)-2-(4-chlorobenzyl)-5-isopropyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol

White powder

89010

96.7% w/w (as total ipconazole), 89.7% w/w (as ipconazole cc),

and 7.0% w/w (as ipconazole ct)

Stability of compound under test conditions:

Results from analytical verification of the royal jelly diet demonstrate that concentrations of the test item as total Ipconazole at 72 hours ranged from 92 to 100% of the nominal concentrations.

Storage conditions of test chemical:

Stored at the test facility at room temperature in a dark, ventilated cabinet in the original container.

**Range finding test yes/no (if yes, describe):** Yes, a preliminary, non-GLP range-finding exposure similar to the definitive exposure was initiated on March 10, 2017 at nominal dose rates of 0.10, 1.0, 10, and 100 µg ai/larva with a negative control and a solvent (acetone) control. One cell plate containing 24 larvae for each dose rate and control was established. Day 7 Mortality was 0% in both the negative and solvent controls as compared to 0% in all treatment groups with the exception of 100% mortality in the highest dose (100 µg ai/larva) test group. Based on these findings, the results of the diet trial, and in consultation with the Study Sponsor, nominal concentrations of 6.3, 13, 25, 50, and 100 µg ai/larva, were selected for the definitive exposure.

**Physicochemical properties of Ipconazole.**

Parameter	Values	Comments
Molecular Weight	Not reported	
Water solubility at 20°C (mg/L)	Not reported	See Reviewer Comments for non-GLP diet trial results
Vapor pressure (torr, at 25°C)	Not reported	
Mean organic carbon partition coefficient $K_{oc}$ (L/kg <sub>oc</sub> )	Not reported	
Log octanol-water partition coefficient Log $K_{ow}$	Not reported	

**A. Test Organisms**

<b>Guideline Criteria</b>	<b>Reported Information</b>	<b>Comments</b>
<b>Species</b>	Honey bee, identified as a mixture of subspecies <i>Apis mellifera lingustica</i> and <i>carnica</i> .	<hr/> OECD recommends European honey bee ( <i>Apis mellifera</i> )
<b>Age at beginning of test</b> Worker bees of uniform age.	1 <sup>st</sup> instar larvae, ≤24 hours old.	<hr/> OECD recommends that on D1 of study, first instar (L1) synchronized larvae (i.e., larvae of the same age) are taken from comb of three colonies.
<b>Source</b>	Honey bee larvae were provided by David Wood, Forsyth, Georgia, from three different hives.	<hr/> OECD recommends larvae are collected from three different colonies.
<b>Were bees from disease-free colonies?</b>	Not reported. The hives were not previously exposed to varroacides or other chemical treatments for at least 4 weeks prior to use in the study.	<hr/> OECD recommends that colonies used to obtain larvae should be adequately fed, health (i.e., as far as disease- and parasite-free), with a known history and physiological status.
<b>Were bees kept in conditions conforming to proper cultural practices?</b>	Not reported but obtained from a beekeeping supply company.	

Guideline Criteria	Reported Information	Comments
<b>Acclimation conditions</b>	Frames containing larvae were brought into the laboratory. The first instar larvae, $\leq 24$ hours old, were removed from the brood cells and each placed into an individual grafting cell. The covered cell plates were placed inside a container located within an environmental chamber for a 3-day acclimation phase. Larvae were fed 20 $\mu\text{L}$ of untreated Diet A upon transfer into the cell plates (D1), not fed on D2, and fed 20 $\mu\text{L}$ of untreated Diet B on D3.	

**B. Test System**

Guideline Criteria	Reported Information	
<b>Test Chambers</b>	Mann Lake (QC-110) single grafting cells, each placed into a well of a 48-well cell culture plate (1.6 mL/well, Corning Costar 3548).	<i>OECD recommends 48-well plate with each well containing a crystal polystyrene grafting cell.</i>
<b>Temperature during exposure</b>	33 to 34 °C	<i>OECD recommends incubator at 34 – 35°C. Deviations may occur but temperature should not be lower than 23°C or higher than 40°C; deviations not last more than 15 minutes once every 24 hrs.</i>
<b>Relative humidity during exposure</b>	94-98%  The outer wells of each cell plate that did not contain larvae were partially filled with deionized water to assist in maintaining a water saturated atmosphere.	<i>OECD recommends use of <math>\text{K}_2\text{SO}_4</math> to maintain water saturated atmosphere.</i>

DP Barcode: 450645

MRID No.: 50451001

Guideline Criteria	Reported Information	
Lighting	Continuous darkness except for approximately 30 minutes each day during observation and renewal of diet	<hr/> OECD recommends that plates should be maintained in darkness.



Guideline Criteria	Reported Information	
<p><b>Feeding</b></p>	<p>On Day 1 (D1), larvae were fed 20 µL of untreated Diet A when transferred into the cell plates. Larvae were not fed on D2 and fed 20 µL of untreated Diet B on D3. Exposure was initiated on D4, when 30 µL of treated Diet C or negative control diet C was added to the larval cell plates. On D5 and D6, all larvae were fed 40 µL and 50 µL, respectively, of untreated Diet C.</p> <p>Diet A<sup>a</sup> (D1): 44.3% weight of fresh royal jelly + 55.7% weight of an aqueous solution containing 1.6% weight of yeast extract + 9.5% weight of glucose + 9.5% weight of fructose.</p> <p>Diet B<sup>a</sup> (D3): 43% weight of fresh royal jelly + 57% weight of an aqueous solution containing 2.3% weight of yeast extract + 11.2% weight of glucose + 11.2% weight of fructose.</p> <p>Diet C<sup>a</sup> (D4-D6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract + 18% weight of glucose + 18% weight of fructose.</p> <p><sup>a</sup> calculated by the reviewer based on diet components provided on page 16 of the MRID.</p>	<p>The study author reported that royal jelly diet preparation ratios were based on modifications from Schmehl <i>et al</i> (2016).</p> <hr/> <p><i>OECD recommends that all larvae are fed once a day. Volume of diet is adjusted each day. Additional food should be added to the cell even if previous allocation has not been totally consumed. Presence of uneaten food at termination of test should be reported.</i></p> <p><i>OECD recommends:</i>  <i>Diet A (D1): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose.</i></p> <p><i>Diet B (D3): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose.</i></p> <p><i>Diet C (from D4 to D6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose.</i></p>

**C. Test Design**

<b>Guideline Criteria</b>	<b>Reported Information</b>	<b>Comments</b>
<b>Nominal dosage levels tested</b>	<p><u>Diet Concentrations:</u> 180, 380, 730, 1500, and 3000 µg ai/g diet</p> <p><u>Dietary Doses:</u> 6.3, 13, 25, 50, and 100 µg ai/larva</p>	<p>The study author calculated the mean measured concentrations from analytical verification of the test item in the larval diet which were subsequently used with the mass of diet provided to each larva to adjust the nominal dose to measured doses.</p> <hr/> <p><i>OECD recommends 5 treatments of increasing test concentrations. Alternatively, when a limit test is performed, a single dose of 100 µg ai/larva or the maximum achievable solubility (whichever is lower).</i></p>
<b>Number of bees exposed per dosage level</b>	12 larvae from three different hives, for a total of 36 larvae per dose level.	<hr/> <p><i>OECD recommends minimum of 12 larvae from each of 3 colonies allocated on the same plate to each treatment, i.e., minimum of 36 larvae per treatment.</i></p>
<b>Other experimental design information</b>	<p>The shape of the larvae at grafting was not reported.</p> <p>Larvae were fed 20-µL of untreated Diet A on D1 when transferred into the cell plates.</p>	<hr/> <p><i>OECD recommends that newly hatched larvae are selected that have not yet formed a "C" shape and randomizing the allocation of larvae into the plates for each colony. On Day 1, larva is</i></p>

Guideline Criteria	Reported Information	Comments
	A 160 mg ai/mL primary stock solution was prepared by bringing 8.1189 g of Ipconazole (7.8510 g as total active ingredient to adjust for purity) to a volume of 50 mL with acetone. The four remaining stock solutions were prepared by serial dilution.	<i>deposited in cell containing 20 µL diet.</i>
<b>Bees randomly or impartially assigned to test groups</b>	Yes, using computer generated random numbers	<i>OECD recommends that each group of a minimum of 12 larvae from each of the three colonies is considered a replicate for a given treatment level and identified as such on the microplate.</i>
<b>Control</b>	Untreated diet  Three replicates with 12 larvae/replicate, for a total of 36 negative control larvae.	<i>OECD recommends 12 larvae x 3 colonies=36 larvae minimum and that control mortality from D4 to D7 should be ≤15%.</i>
<b>Solvent control</b>	Untreated diet with acetone  Three replicates with 12 larvae/replicate, for a total of 36 solvent control larvae.	<i>OECD recommends maximum of 5%.</i>
<b>Reference Toxicant</b>	The reference item, Dimethoate was tested at a nominal 8.8 µg ai/larva.  Three replicates with 12 larvae per replicate, for a total of 36 reference larvae.	<i>OECD recommends technical grade dimethoate at dose of 8.8 ± 0.5 µg a.i./larva. Mortality should be ≥50% at D7 for toxic reference.</i>
<b>Total observation period and frequency of interim observations</b>	Mortality assessments were conducted and recorded daily from Day 4 through 7.	<i>OECD recommends that following chemical exposure on D4, mortalities are</i>

Guideline Criteria	Reported Information	Comments
	Uneaten diet and health observations were recorded on Day 7 (test termination).	<i>checked at time of feeding on D5, D6 and D7 (test termination). Other observations including presence of uneaten food on D7 should be qualitatively reported.</i>

**12. REPORTED RESULTS:**

Guideline Criteria	Reported Information
<b>Quality Assurance, No Data Confidentiality Claim and GLP compliance statements were included in the report?</b>	Yes. The data and report were produced and compiled in accordance with all pertinent U.S. Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Standards as set forth under the Federal Insecticide, Fungicide, and Rodenticide Act (40 CFR, Part 160), and as accepted by OECD Principles of GLP (1998) with two exceptions: 1) Routine water and royal jelly diet contaminant screening analyses for pesticides, PCB's, toxic metals, and antibiotics were conducted using standard U.S. EPA procedures by GeoLabs, Inc., Braintree, Massachusetts and the U.S. Department of Agriculture, Gastonia, North Carolina, and are considered facility records under Smithers Viscient's Standard Operating Procedures and 2) The reference toxicant, Dimethoate, was purchased from a commercial supplier and was not characterized according to GLP Standards.
<b>Observed adverse effects on bees at respective dosages</b>	<p>At test termination (Day 7), cumulative larval mortality ranged from 8 to 100% in the treatment groups. 100% of larvae in the highest dose treatment group (100 µg ai/larva) had died by Day 7.</p> <p>On Day 7, 1 larva in the control and 3 larvae in the solvent control were observed to be small in size, as compared to 4, 4, 7, and 4 larvae observed as small in size in the 5.7, 12, 25, and 49 µg ai/larva treatment</p>

Guideline Criteria	Reported Information
	groups, respectively. All other surviving larvae fed treated and control diets were observed to be healthy.  On Day 7, uneaten food was present in 11 replicates in both the negative and solvent controls, as compared to uneaten food present in 13, 12, 12, and 4 replicates in the 5.7, 12, 25, and 49 µg ai/larva treatment groups, respectively.
<b>Control and Solvent Control Mortality</b>	<u>Negative Control</u> : 0% <u>Solvent Control</u> : 0%
<b>Were raw data included?</b>	No, only group summary tables were provided.
<b>Analytical Verification?</b>	Yes, all stock solution, royal jelly diet, and QC samples were analyzed for total Ipconazole concentration (as the sum of the Ipconazole cc and ct isomers which were quantified separately) by liquid chromatography with mass spectrometry detection (LC/MS/MS) based on methodology validated at Smithers Viscient.

### Mortality and Observations:

At test termination (Day 7), cumulative larval mortality was 0% in both the negative and solvent controls, respectively, as compared to mortality ranging from 8 to 100% in the treatment groups (Table 1).

On Day 7, 1 larva in the control and 3 larvae in the solvent control were observed to be small in size, as compared to 4, 4, 7, and 4 larvae observed as small in size in the 5.7, 12, 25, and 49 µg ai/larva treatment groups, respectively. All other surviving larvae fed treated and control diets were observed to be healthy.

On Day 7, uneaten food was present in 11 replicates in both the negative and solvent controls, as compared to uneaten food present in 13, 12, 12, and 4 replicates in the 5.7, 12, 25, and 49 µg ai/larva treatment groups, respectively.

The reference toxicant (Dimethoate) tested at a nominal dose of 8.8 µg ai/larva caused mortality of 61% by Day 7.

**Table 1. Cumulative honey bee larval mortality data after single dietary exposure (dose) to Ipconazole<sup>a</sup>.**

Measured Dose (Mean-Measured Concentration)	Number Exposed	Day4 % (# dead)	Day 5 % (# dead)	Day 6 % (# dead)	Day 7 % (# dead)
Negative Control	36	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	36	0 (0)	0 (0)	0 (0)	0 (0)
5.7 µg ai/larva (170 µg ai/g diet)	36	0 (0)	0 (0)	3 (1)	8 (3)
12 µg ai/larva (360 µg ai/g diet)	36	0 (0)	0 (0)	6 (2)	17 (6)
25 µg ai/larva (730 µg ai/g diet)	36	0 (0)	0 (0)	0 (0)	47 (17)
49 µg ai/larva (1400 µg ai/g diet)	36	0 (0)	0 (0)	33 (12)	89 (32)
100 µg ai/larva (3100 µg ai/g diet)	36	0 (0)	14 (5)	58 (21)	100 (36)
Nominal Dimethoate 8.8 µg ai/larva	36	0 (0)	8 (3)	28 (10)	61 (22)

<sup>a</sup> Mortality based on survival data reported on page 31 of the MRID.

### Reported Statistical Analysis:

When sufficient mortality occurred, the calculated dose (µg ai/larva) and measured diet concentration (µg ai/g diet) tested, combined with the corresponding mortality data, were used to estimate the 72-hour LD<sub>50</sub> and LC<sub>50</sub>, respectively. The LD<sub>50</sub>/LC<sub>50</sub> values were determined by Trimmed Spearman-Kärber Estimates.

CETIS<sup>TM</sup> version 1.8 (2013) was used to perform all statistical analyses.

The study author reported the following endpoints, based on mean-measured concentrations and adjusted/measured dose:

**72-hr LD<sub>50</sub>:** 24 µg ai/larva      95% C.I.: 20 – 29 µg ai/larva

**72-hr LC<sub>50</sub>:** 690 µg ai/g diet      **95% C.I.:** 570 – 840 µg ai/g diet

### Reviewer's Statistical Analysis:

Statistical method: Mortality data were analyzed using CETIS statistical software version 1.9.5.3 with database backend settings implemented by EFED on 7/25/17. Two test records were established named "50451001 dc" and "50451001 dd" corresponding to measured diet concentrations and measured dietary doses, respectively.

Negative and solvent control data were not significantly different based on Fisher's Exact test. All further hypothesis testing was conducted comparing treatment data to negative control data only.

A Fisher Exact/Bonferroni-Holm test ( $\alpha = 0.05$ ) was used to compare treatment data to negative control data. Linear regression was used to estimate LC/LD<sub>50</sub> values with defined 95% confidence intervals.

	<b>Mortality</b>
Diet Concentration (µg ai/g diet)	LC <sub>50</sub> : 649 95% CI: 540 to 779 Slope: 3.1 (2.4 to 3.9) NOAEC: 170 LOAEC: 360
Dietary Dose (µg ai/larva)	LD <sub>50</sub> : 22 95% CI: 18 to 27 Slope: 3.1 (2.4 to 3.8) NOAEL: 5.7 LOAEL: 12

### 13. REVIEWER'S COMMENTS:

The reviewer's LC/LD<sub>50</sub> estimates were lower than the study author's, likely due to use of linear regression (used by the reviewer) for analysis and reporting versus the use of the Trimmed Spearman-Kärber method (used by the study author). The study author did not determine NOAEC/LOAEC values. The reviewer's findings are presented in the Conclusions section of this DER.

Replicate data were not provided, so the reviewer performed statistical analyses using one replicate per treatment level and control group with 36 larvae per level.

The study was initiated on April 21, 2017. The acclimation phase took place from April 28 to 30, 2017 and exposure took place from May 1 to 4, 2017.

Prior to conducting the definitive exposure, a non-GLP diet trial was conducted under Smithers Viscient Study Number 11106.6107. The objective of the trial was to determine the solubility, homogeneity, and 4-day stability of Ipconazole in royal jelly diet at relevant dose rates. Based on the analytical results and observations of the royal jelly diet, it was determined that homogenous concentrations of Ipconazole could be prepared in diet up to a dose of 100 µg ai/larva (3000 µg ai/g) during definitive testing. It was also determined that the concentration of Ipconazole in refrigerated royal jelly diet remained stable for at least four days. Using the functionally soluble portion of the sucrose diet dosed at the equivalency of 100 µg ai/bee/day yielded mean recoveries of 32% in the 50% sucrose solution diet and 22% in the 67% sucrose solution diet. These represent the maximum achievable concentration (equivalent to 32 µg ai/bee/day) in 50% sucrose solution diet during definitive testing.

Both validity criteria for the OECD guideline used were met in this study:

- 1) Cumulative larval mortality from D4 to D7  $\leq$  15% across control replicates.
- 2) Larval mortality in the reference chemical treatment (Dimethoate) was  $\geq$  50% at D7.

#### **14. REVIEWER'S CONCLUSIONS:**

This study is **scientifically sound** and is classified as **acceptable**. The NOAEC and 72-hour LC<sub>50</sub> were 170 and 649 µg ai/g diet, respectively, corresponding to a NOAEL and LD<sub>50</sub> of 5.7 and 22 µg ai/larva, respectively.

#### **15. REFERENCES:**

- Ives, M., 2013. CETIS, Comprehensive Environmental Toxicity Information System™, User's Guide. Tidepool Scientific Software, McKinleyville, California.
- Schmehl, D. R., H. V. V. Tome, A. N. Mortensen, G. F. Martins, and J. D. Ellis, 2016. Protocol For the in vitro rearing of honey bee (*Apis mellifera* L.) workers. Journal of Apicultural Research 55: 113-129.

All other references were standard guidelines or methodologies.



# CETIS Summary Report

Report Date: 13 May-19 12:28 (p 1 of 1)  
Test Code/ID: 50451001 dc / 08-4391-8088

OECD TG237 Honey bee Larval Acute Toxicity				Smithers Viscient	
Batch ID:	19-6917-4491	Test Type:	OECD 237 Honeybee Acute Larval	Analyst:	
Start Date:	01 May-17	Protocol:	OECD 237: Acute Larval Single Exposure	Diluent:	
Ending Date:	04 May-17	Species:	Apis mellifera	Brine:	
Test Length:	72h	Taxon:		Source:	David Wood, Forsyth, GA
				Age:	<24
Sample ID:	07-4231-7696	Code:	50451001 dc	Project:	Fungicide
Sample Date:	01 May-17	Material:	Ipconazole	Source:	Kureha Corporation
Receipt Date:	04 May-17	CAS (PC):		Station:	
Sample Age:	n/a	Client:	CDM Smith		

125618 50451001, mean-measured concentration (ug ai/g diet), stats performed by A. Graff

## Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
18-0902-2951	72h Mortality Rate	Fisher Exact Test	1.0000	Solvent Blank passed 72h mortality rate	1

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	TU	PMSD	S
12-0521-1737	72h Mortality Rate	Fisher Exact/Bonferroni-Holm Test	✓ 170	360	247.4		n/a	1
16-5096-6489	72h Mortality Rate	Fisher Exact/Bonferroni-Holm Test	✓ 170	360	247.4		n/a	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg ai/g	95% LCL	95% UCL	TU	S
08-8420-6791	72h Mortality Rate	GLM: Log-Normal (Probit)	LC1	117	65.1	169		1
			LC5	193	125	257		
			LC10	252	175	323		
			LC15	302	220	378		
			LC20	349	263	429		
			LC25	395	305	479		
			LC40	539	440	644		
04-5317-7479	72h Mortality Rate	Trimmed Spearman-Kärber	✓ LC50	649	540	779		1
			LC50	694	575	839		

## 72h Mortality Rate Summary

Conc-µg ai/g	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	1	0.000			0.000	0.000	0.000	0.000		0.00%
0	N	1	0.000			0.000	0.000	0.000	0.000		0.00%
170		1	0.083			0.083	0.083	0.000	0.000	0.00%	8.33%
360		1	0.167			0.167	0.167	0.000	0.000	0.00%	16.67%
730		1	0.472			0.472	0.472	0.000	0.000	0.00%	47.22%
1400		1	0.889			0.889	0.889	0.000	0.000	0.00%	88.89%
3100		1	1.000			1.000	1.000	0.000	0.000	0.00%	100.00%

## 72h Mortality Rate Detail

Conc-µg ai/g	Code	Rep 1
0	S	0.000
0	N	0.000
170		0.083
360		0.167
730		0.472
1400		0.889
3100		1.000

# CETIS Summary Report

Report Date: 13 May-19 12:30 (p 1 of 1)  
 Test Code/ID: 50451001 dd / 13-5007-9230

OECD TG237 Honey bee Larval Acute Toxicity				Smithers Viscient	
Batch ID:	16-0218-7237	Test Type:	OECD 237 Honeybee Acute Larval	Analyst:	
Start Date:	01 May-17	Protocol:	OECD 237: Acute Larval Single Exposure	Diluent:	
Ending Date:	04 May-17	Species:	Apis mellifera	Brine:	
Test Length:	72h	Taxon:		Source:	David Wood, Forsyth, GA    Age: <24
Sample ID:	08-8329-8773	Code:	50451001 dd	Project:	Fungicide
Sample Date:	01 May-17	Material:	Ipconazole	Source:	Kureha Corporation
Receipt Date:	04 May-17	CAS (PC):		Station:	
Sample Age:	n/a	Client:	CDM Smith		

125618 50451001, measured dose (ug ai/larva), stats performed by A. Graff

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	ug ai/larv	95% LCL	95% UCL	TU	S
10-2685-3720	72h Mortality Rate	GLM: Log-Normal (Probit)	LC1	3.89	2.17	5.65		1
			LC5	6.46	4.17	8.64		
			LC10	8.48	5.87	10.9		
			LC15	10.2	7.39	12.8		
			LC20	11.8	8.84	14.5		
			LC25	13.3	10.3	16.2		
			LC40	18.3	14.9	21.9		
			✓ LC50	22.1	18.3	26.5		
16-3118-2307	72h Mortality Rate	Trimmed Spearman-Kärber	LC50	23.7	19.6	28.8		1

## 72h Mortality Rate Summary

Conc-ug ai/larv	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	1	0.000			0.000	0.000	0.000	0.000		0.00%
0	N	1	0.000			0.000	0.000	0.000	0.000		0.00%
5.7		1	0.083			0.083	0.083	0.000	0.000	0.00%	8.33%
12		1	0.167			0.167	0.167	0.000	0.000	0.00%	16.67%
25		1	0.472			0.472	0.472	0.000	0.000	0.00%	47.22%
49		1	0.889			0.889	0.889	0.000	0.000	0.00%	88.89%
100		1	1.000			1.000	1.000	0.000	0.000	0.00%	100.00%

## 72h Mortality Rate Detail

Conc-ug ai/larv	Code	Rep 1
0	S	0.000
0	N	0.000
5.7		0.083
12		0.167
25		0.472
49		0.889
100		1.000

# CETIS Analytical Report

Report Date: 13 May-19 12:28 (p 1 of 3)  
Test Code/ID: 50451001 dc / 08-4391-8088

## OECD TG237 Honey bee Larval Acute Toxicity Smithers Viscient

<b>Analysis ID:</b> 16-5096-6489	<b>Endpoint:</b> 72h Mortality Rate	<b>CETIS Version:</b> CETISv1.9.5
<b>Analyzed:</b> 13 May-19 12:25	<b>Analysis:</b> STP 2xK Contingency Tables	<b>Status Level:</b> 1
<b>Batch ID:</b> 19-6917-4491	<b>Test Type:</b> OECD 237 Honeybee Acute Larval	<b>Analyst:</b>
<b>Start Date:</b> 01 May-17	<b>Protocol:</b> OECD 237: Acute Larval Single Exposure	<b>Diluent:</b>
<b>Ending Date:</b> 04 May-17	<b>Species:</b> Apis mellifera	<b>Brine:</b>
<b>Test Length:</b> 72h	<b>Taxon:</b>	<b>Source:</b> David Wood, Forsyth, GA <b>Age:</b> <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C < T	170	360	247.4	

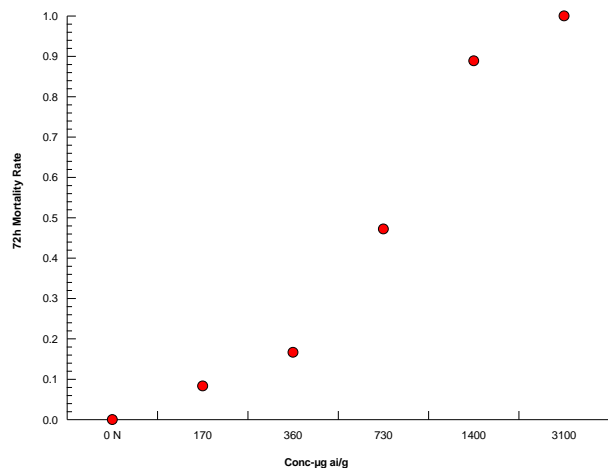
### Fisher Exact/Bonferroni-Holm Test

Control	vs	Group	Test Stat	P-Type	P-Value	Decision(α:5%)
Negative Control		170	0.120	Exact	0.1197	Non-Significant Effect
		360*	0.012	Exact	0.0249	Significant Effect
		730*	0.000	Exact	1.9E-06	Significant Effect
		1400*	0.000	Exact	8.3E-16	Significant Effect
		3100*	0.000	Exact	1.1E-20	Significant Effect

### Data Summary

Conc-µg ai/g	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	N	36	0	36	1	0	
170		33	3	36	0.917	0.0833	
360		30	6	36	0.833	0.167	
730		19	17	36	0.528	0.472	
1400		4	32	36	0.111	0.889	
3100		0	36	36	0	1	

### Graphics



# CETIS Analytical Report

Report Date: 13 May-19 12:28 (p 2 of 3)  
Test Code/ID: 50451001 dc / 08-4391-8088

## OECD TG237 Honey bee Larval Acute Toxicity Smithers Viscient

<b>Analysis ID:</b> 12-0521-1737	<b>Endpoint:</b> 72h Mortality Rate	<b>CETIS Version:</b> CETISv1.9.5
<b>Analyzed:</b> 13 May-19 12:25	<b>Analysis:</b> STP 2xK Contingency Tables	<b>Status Level:</b> 1
<b>Batch ID:</b> 19-6917-4491	<b>Test Type:</b> OECD 237 Honeybee Acute Larval	<b>Analyst:</b>
<b>Start Date:</b> 01 May-17	<b>Protocol:</b> OECD 237: Acute Larval Single Exposure	<b>Diluent:</b>
<b>Ending Date:</b> 04 May-17	<b>Species:</b> Apis mellifera	<b>Brine:</b>
<b>Test Length:</b> 72h	<b>Taxon:</b>	<b>Source:</b> David Wood, Forsyth, GA <b>Age:</b> <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C < T	170	360	247.4	

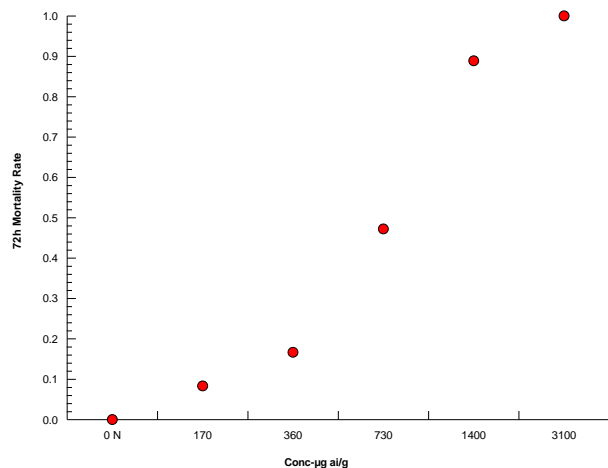
### Fisher Exact/Bonferroni-Holm Test

Control	vs	Group	Test Stat	P-Type	P-Value	Decision(α:5%)
Negative Control		170	0.120	Exact	0.1197	Non-Significant Effect
		360*	0.012	Exact	0.0249	Significant Effect
		730*	0.000	Exact	1.9E-06	Significant Effect
		1400*	0.000	Exact	8.3E-16	Significant Effect
		3100*	0.000	Exact	1.1E-20	Significant Effect

### Data Summary

Conc-µg ai/g	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	N	36	0	36	1	0	
170		33	3	36	0.917	0.0833	
360		30	6	36	0.833	0.167	
730		19	17	36	0.528	0.472	
1400		4	32	36	0.111	0.889	
3100		0	36	36	0	1	

### Graphics



# CETIS Analytical Report

Report Date: 13 May-19 12:28 (p 3 of 3)  
Test Code/ID: 50451001 dc / 08-4391-8088

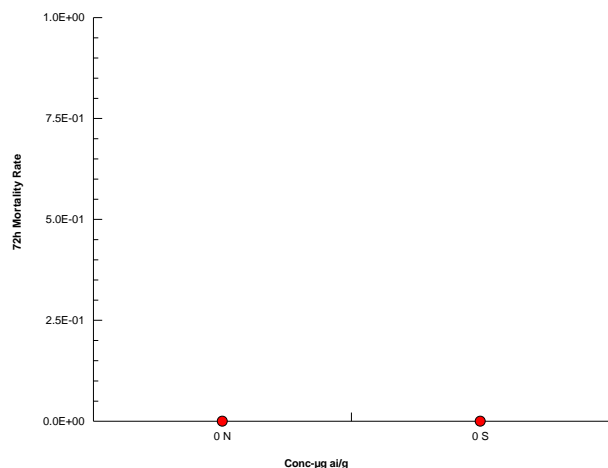
OECD TG237 Honey bee Larval Acute Toxicity			Smithers Viscient
Analysis ID: 18-0902-2951	Endpoint: 72h Mortality Rate	CETIS Version: CETISv1.9.5	
Analyzed: 13 May-19 12:26	Analysis: Single 2x2 Contingency Table	Status Level: 1	
Batch ID: 19-6917-4491	Test Type: OECD 237 Honeybee Acute Larval	Analyst:	
Start Date: 01 May-17	Protocol: OECD 237: Acute Larval Single Exposure	Diluent:	
Ending Date: 04 May-17	Species: Apis mellifera	Brine:	
Test Length: 72h	Taxon:	Source: David Wood, Forsyth, GA	Age: <24

Data Transform	Alt Hyp	Comparison Result
Untransformed	C <> T	Solvent Blank passed 72h mortality rate

Fisher Exact Test						
Control	vs	Control	Test Stat	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	1.000	Exact	1.0000	Non-Significant Effect

Data Summary							
Conc-µg ai/g	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	S	36	0	36	1	0	
0	N	36	0	36	1	0	

## Graphics



## CETIS Analytical Report

Report Date: 13 May-19 12:27 (p 1 of 2)  
 Test Code/ID: 50451001 dc / 08-4391-8088

OECD TG237 Honey bee Larval Acute Toxicity						Smithers Viscient	
Analysis ID:	08-8420-6791	Endpoint:	72h Mortality Rate	CETIS Version:	CETISv1.9.5		
Analyzed:	13 May-19 12:26	Analysis:	Linear Regression (GLM)	Status Level:	1		
Batch ID:	19-6917-4491	Test Type:	OECD 237 Honeybee Acute Larval	Analyst:			
Start Date:	01 May-17	Protocol:	OECD 237: Acute Larval Single Exposure	Diluent:			
Ending Date:	04 May-17	Species:	Apis mellifera	Brine:			
Test Length:	72h	Taxon:		Source:	David Wood, Forsyth, GA	Age:	<24

## Linear Regression Options

Model Name	Link Function	Threshold Option	Thresh	Optimize	Pooled	Het Corr	Weighted
Log-Normal (Probit)	$\eta = \text{inv } \Phi[\pi]$	Zero Threshold	0	No	No	No	Yes

## Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	PMSD	F Stat	P-Value	Decision( $\alpha$ :5%)
5	-9.38	28.8	22	2.81	0.32	0.988				Lack of Fit Not Tested

## Point Estimates

Level	$\mu$ g ai/g	95% LCL	95% UCL
LC1	117	65.1	169
LC5	193	125	257
LC10	252	175	323
LC15	302	220	378
LC20	349	263	429
LC25	395	305	479
LC40	539	440	644
LC50	649	540	779

## Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision( $\alpha$ :5%)
Intercept	-8.78	1.08	-10.9	-6.65	-8.09	0.0039	Significant Parameter
Slope	3.12	0.382	2.37	3.87	8.17	0.0038	Significant Parameter

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision( $\alpha$ :5%)
Model	582	582	1	334	3.6E-04	Significant
Residual	5.23	1.74	3			

## Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision( $\alpha$ :5%)
Model Fit	Likelihood Ratio GOF Test	5.19	7.81	0.1584	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	5.23	7.81	0.1555	Non-Significant Heterogeneity
Distribution	Shapiro-Wilk W Normality Test	0.935	0.34	0.6314	Normal Distribution

## 72h Mortality Rate Summary

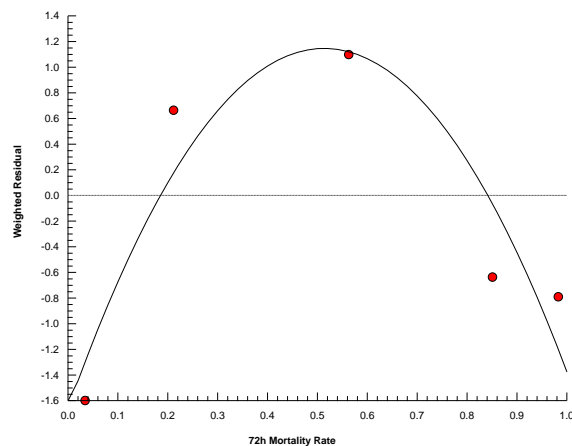
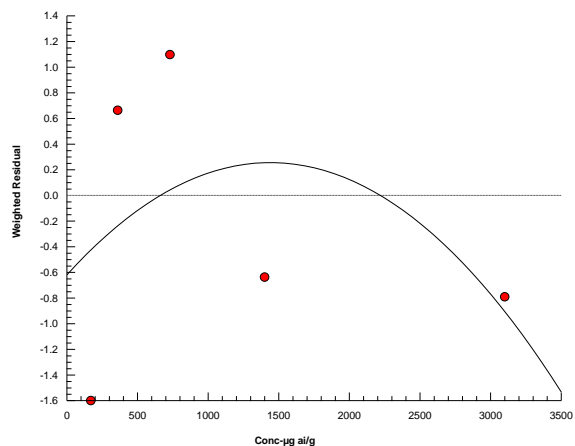
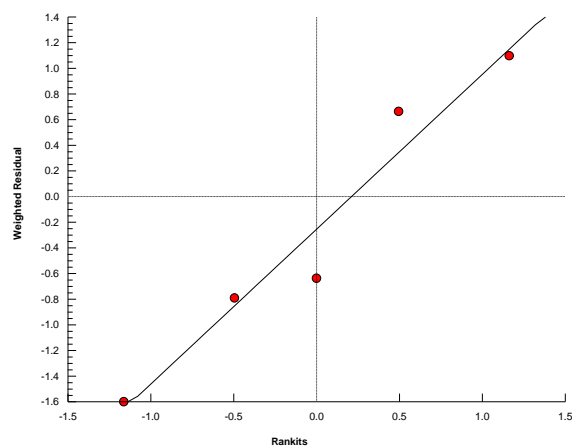
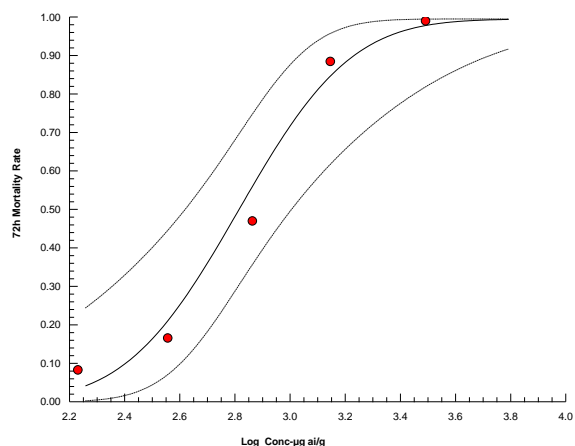
		Calculated Variate(A/B)									
Conc- $\mu$ g ai/g	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
170		1	0.083	0.083	0.083	0.000	0.000	0.00%	0.0%	3	36
360		1	0.167	0.167	0.167	0.000	0.000	0.00%	9.09%	6	36
730		1	0.472	0.472	0.472	0.000	0.000	0.00%	42.4%	17	36
1400		1	0.889	0.889	0.889	0.000	0.000	0.00%	87.9%	32	36
3100		1	1.000	1.000	1.000	0.000	0.000	0.00%	100.0%	36	36

## OECD TG237 Honey bee Larval Acute Toxicity

Smithers Viscient

Analysis ID: 08-8420-6791  
Analyzed: 13 May-19 12:26Endpoint: 72h Mortality Rate  
Analysis: Linear Regression (GLM)CETIS Version: CETISv1.9.5  
Status Level: 1

## Graphics

Log-Normal:  $\text{inv } \Phi[\pi] = \alpha + \beta \cdot \log[x]$ 

## CETIS Analytical Report

Report Date: 13 May-19 12:30 (p 1 of 2)  
 Test Code/ID: 50451001 dd / 13-5007-9230

OECD TG237 Honey bee Larval Acute Toxicity				Smithers Viscient			
Analysis ID:	10-2685-3720	Endpoint:	72h Mortality Rate	CETIS Version:	CETISv1.9.5		
Analyzed:	13 May-19 12:30	Analysis:	Linear Regression (GLM)	Status Level:	1		
Batch ID:	16-0218-7237	Test Type:	OECD 237 Honeybee Acute Larval	Analyst:			
Start Date:	01 May-17	Protocol:	OECD 237: Acute Larval Single Exposure	Diluent:			
Ending Date:	04 May-17	Species:	Apis mellifera	Brine:			
Test Length:	72h	Taxon:		Source:	David Wood, Forsyth, GA	Age:	<24

## Linear Regression Options

Model Name	Link Function	Threshold Option	Thresh	Optimize	Pooled	Het Corr	Weighted
Log-Normal (Probit)	$\eta = \text{inv } \Phi[\pi]$	Zero Threshold	0	No	No	No	Yes

## Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	PMSD	F Stat	P-Value	Decision( $\alpha$ :5%)
5	-9.47	28.9	22.2	1.34	0.324	0.988				Lack of Fit Not Tested

## Point Estimates

Level	ug ai/larv	95% LCL	95% UCL
LC1	3.89	2.17	5.65
LC5	6.46	4.17	8.64
LC10	8.48	5.87	10.9
LC15	10.2	7.39	12.8
LC20	11.8	8.84	14.5
LC25	13.3	10.3	16.2
LC40	18.3	14.9	21.9
LC50	22.1	18.3	26.5

## Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision( $\alpha$ :5%)
Intercept	-4.14	0.519	-5.16	-3.13	-7.99	0.0041	Significant Parameter
Slope	3.08	0.371	2.36	3.81	8.3	0.0037	Significant Parameter

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision( $\alpha$ :5%)
Model	577	577	1	330	3.6E-04	Significant
Residual	5.25	1.75	3			

## Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision( $\alpha$ :5%)
Model Fit	Likelihood Ratio GOF Test	5.37	7.81	0.1469	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	5.25	7.81	0.1544	Non-Significant Heterogeneity
Distribution	Shapiro-Wilk W Normality Test	0.955	0.34	0.7759	Normal Distribution

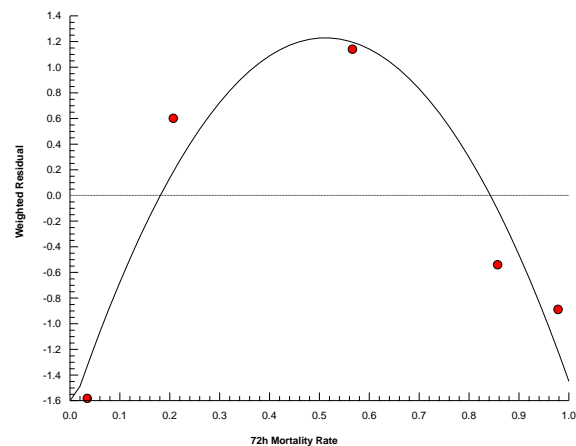
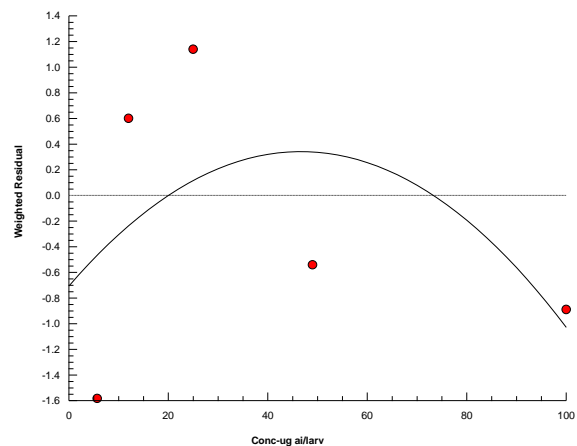
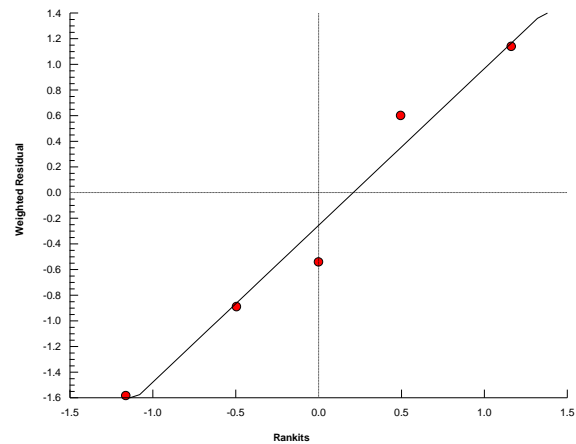
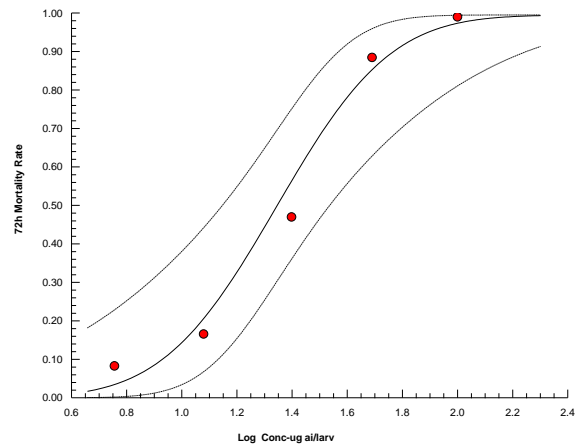
## 72h Mortality Rate Summary

		Calculated Variate(A/B)									
Conc-ug ai/larv	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
5.7		1	0.083	0.083	0.083	0.000	0.000	0.00%	0.0%	3	36
12		1	0.167	0.167	0.167	0.000	0.000	0.00%	9.09%	6	36
25		1	0.472	0.472	0.472	0.000	0.000	0.00%	42.4%	17	36
49		1	0.889	0.889	0.889	0.000	0.000	0.00%	87.9%	32	36
100		1	1.000	1.000	1.000	0.000	0.000	0.00%	100.0%	36	36



Analysis ID:	10-2685-3720	Endpoint:	72h Mortality Rate	CETIS Version:	CETISv1.9.5
Analyzed:	13 May-19 12:30	Analysis:	Linear Regression (GLM)	Status Level:	1

Graphics Log-Normal:  $\text{inv } \Phi[\pi] = \alpha + \beta \cdot \log[x]$



# CETIS Analytical Report

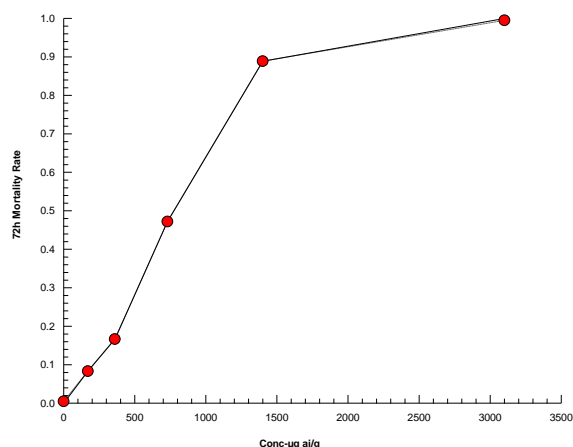
Report Date: 13 May-19 12:27 (p 1 of 1)  
Test Code/ID: 50451001 dc / 08-4391-8088

OECD TG237 Honey bee Larval Acute Toxicity				Smithers Viscient			
Analysis ID:	04-5317-7479	Endpoint:	72h Mortality Rate	CETIS Version:	CETISv1.9.5		
Analyzed:	13 May-19 12:26	Analysis:	Trimmed Spearman-Kärber	Status Level:	1		
Batch ID:	19-6917-4491	Test Type:	OECD 237 Honeybee Acute Larval	Analyst:			
Start Date:	01 May-17	Protocol:	OECD 237: Acute Larval Single Exposure	Diluent:			
Ending Date:	04 May-17	Species:	Apis mellifera	Brine:			
Test Length:	72h	Taxon:		Source:	David Wood, Forsyth, GA	Age:	<24

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	8.33%	2.84	0.0411	694	575	839

72h Mortality Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg ai/g	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	A/B	Mean	%Effect
0	N	1	0.000	0.000	0.000	0.000		0.0%	0/36	0	0.0%
170		1	0.083	0.083	0.083	0.000	0.00%	8.33%	3/36	0.0833	8.33%
360		1	0.167	0.167	0.167	0.000	0.00%	16.7%	6/36	0.167	16.7%
730		1	0.472	0.472	0.472	0.000	0.00%	47.2%	17/36	0.472	47.2%
1400		1	0.889	0.889	0.889	0.000	0.00%	88.9%	32/36	0.889	88.9%
3100		1	1.000	1.000	1.000	0.000	0.00%	100.0%	36/36	1	100.0%

## Graphics



# CETIS Analytical Report

Report Date: 13 May-19 12:30 (p 1 of 1)  
 Test Code/ID: 50451001 dd / 13-5007-9230

OECD TG237 Honey bee Larval Acute Toxicity				Smithers Viscient	
Analysis ID:	16-3118-2307	Endpoint:	72h Mortality Rate	CETIS Version:	CETISv1.9.5
Analyzed:	13 May-19 12:30	Analysis:	Trimmed Spearman-Kärber	Status Level:	1
Batch ID:	16-0218-7237	Test Type:	OECD 237 Honeybee Acute Larval	Analyst:	
Start Date:	01 May-17	Protocol:	OECD 237: Acute Larval Single Exposure	Diluent:	
Ending Date:	04 May-17	Species:	Apis mellifera	Brine:	
Test Length:	72h	Taxon:		Source:	David Wood, Forsyth, GA
				Age:	<24

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	8.33%	1.38	0.042	23.7	19.6	28.8

72h Mortality Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-ug ai/larv	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	A/B	Mean	%Effect
0	N	1	0.000	0.000	0.000	0.000		0.0%	0/36	0	0.0%
5.7		1	0.083	0.083	0.083	0.000	0.00%	8.33%	3/36	0.0833	8.33%
12		1	0.167	0.167	0.167	0.000	0.00%	16.7%	6/36	0.167	16.7%
25		1	0.472	0.472	0.472	0.000	0.00%	47.2%	17/36	0.472	47.2%
49		1	0.889	0.889	0.889	0.000	0.00%	88.9%	32/36	0.889	88.9%
100		1	1.000	1.000	1.000	0.000	0.00%	100.0%	36/36	1	100.0%

